A transformational one-step library preparation method for multiplexed plasmid and amplicon sequencing

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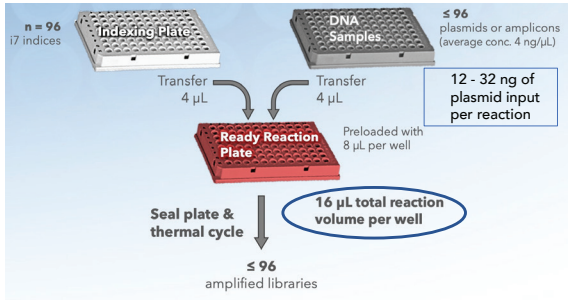
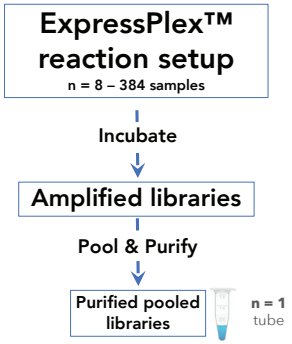
Introduction

Conventional library preparation methods demand multiple incubations interrupted by cumbersome pipetting steps. A transformational new library prep technology from seqWell enables barcoding and amplification of 8 - 384 dual-indexed libraries in 90 minutes in 16 µl "one-pot" reactions, while simultaneously auto-normalizing library read-count and insert size. The simplicity of the ExpressPlex™ workflow makes it uniquely well-suited for manual, automated and miniaturized library prep from plasmids and PCR products. To demonstrate miniaturization, we prepared plasmid libraries on the SPT LabTech mosquito® HV at 3,200 nanoliters per reaction in 384-well PCR plates, and the sequencing data were used for *de novo*, full plasmid assembly.

ExpressPlex™ library prep workflows

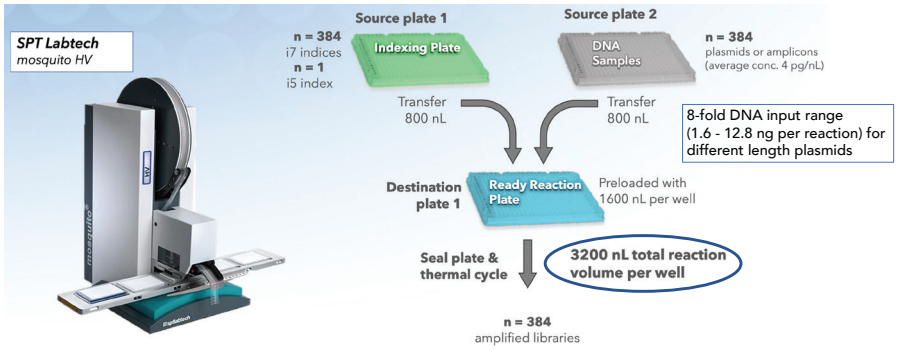
A. Manual / Automated 96-plex ExpressPlex™ workflow





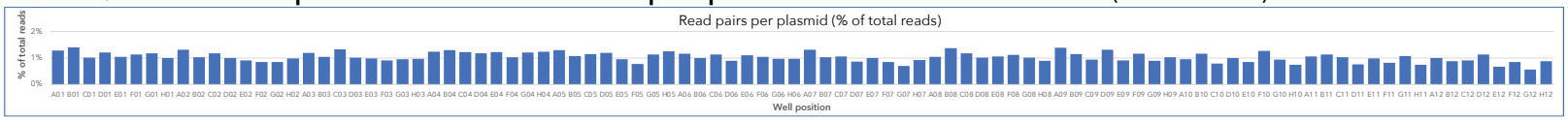
(Total elapsed time: 90 min)

B. Miniaturized 384-plex ExpressPlex™ workflow



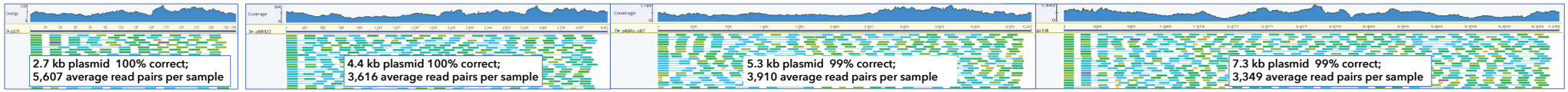
Results

A. Manual / automated ExpressPlex™ workflow for 96-plex plasmid libraries : Read balance (c.v. = 16.6%)



1.04%	Average reads per plasmid
0.17%	standard deviation
16.6%	c.v.

B. Miniaturized ExpressPlex™ 384-plex libraries with variable DNA input: Typical depth of coverage profiles and *de novo* plasmid assembly success rate



Summary: 382 out of 384 plasmids resulted in circularized *de novo* assemblies that were 100% concordant with their known references (i.e., 99.5% overall success rate).

Conclusions

- Full-volume 96-plex ExpressPlex™ reactions (16 µL) set-up manually or with standard automated liquid handlers produce a uniform number of sequencing reads per plasmid (c.v. 16.6%).
- 384 miniaturized ExpressPlex™ reactions (3,200 nL) set-up with an 8-fold DNA input range (1.6 – 12.8 ng per reaction) resulted in a very high *de novo* plasmid assembly success rate (99.5%), even when sequenced to modest depth (3.3 – 5.6 K read pairs per sample) on a 2 x 151 cycle MiSeq Micro v2 kit.
- These results suggest that the *de novo* plasmid assembly success rate would be in the vicinity of 99.5%, if 768 ExpressPlex™ plasmid libraries (8 x 96-well plates) were pooled and sequenced on a 300 cycle MiSeq Micro v2 kit, or if 2,304 ExpressPlex™ plasmid libraries (6 x 384-well plates) were pooled and sequenced on a 300 cycle MiSeq v2 kit.